Sodium-Dependent SLC Transporter Assay

Catalog No. 8000-10

Introduction

Solute carrier (SLC) transporters represent one of the largest families of integral membrane proteins. They play important roles in cellular homeostasis by mediating the uptake and efflux of a wide range of substrates, metabolites, and neurotransmitters. With over 400 members identified in humans, these transporters are critical for nutrient absorption, waste removal, and the regulation of neuronal signaling. Disruptions or mutations in these transporters are increasingly recognized as contributing factors to a wide range of pathologies such as hypertension, epilepsy, and metabolic disorders. As a result, SLC transporters are at the forefront of drug discovery and therapeutic research, with ongoing efforts to learn their mechanisms and develop compounds that modulate their activity.

Sodium-dependent SLC transporters harness the energy stored in the sodium electrochemical gradient to drive the transport of substrates. As these transporters move their substrates into the cell, sodium ions are carried along, leading to an increase in the intracellular sodium concentration. ION Biosciences' Sodium-Dependent SLC Transporter Assay utilizes ION Natrium Green 2 (ING-2 AM), a sodium-sensitive fluorescent indicator, to detect SLC transporter mediated sodium influx. This assay kit provides a sensitive, robust, and high-throughput method to measure SLC transporter activity. This platform generates EC_{50} and IC_{50} values comparable to those from electrophysiology by precisely quantifying transporter activity. It enables efficient screening of novel compounds and rapid identification of therapeutic candidates targeting SLC transporters.

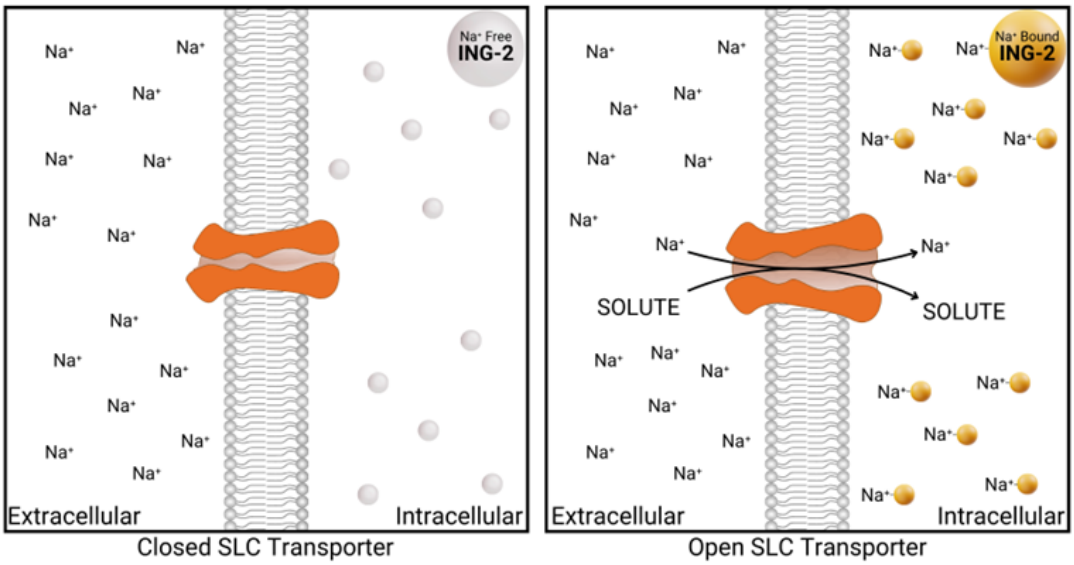

Figure 1. Sodium-dependent SLC Transporter Assay Principle. Cells expressing SLC transporters are incubated in a dye loading solution containing ING-2, a fluorescent indicator that senses sodium. When the substrate is introduced, the SLC transporter moves both the substrate and extracellular sodium into the cell. The increase in intracellular sodium is detected as a change in fluorescence, providing a measurable signal of transporter activity for analysis.

Storage and Stability

Upon receipt, store components at the temperatures indicated on each label. Kit components are stable[1] for up to 12 months from date of shipment when stored as directed.

Table 1	Kit Contents	*Flex* Kit Cat# 8000-10		
Label	Name	Size	Qty	Storage
Reagent A	ING-2 AM	50 µg Vial	10	-20°C
Reagent B	DMSO[1]	225 µL Vial	1	-20°C
Reagent C	50X DySolv	4 mL Bottle	1	4°C
Reagent D	10X SLC Sodium Assay Buffer	30 mL Bottle	1	4°C
Reagent E	50X TRS[2]	4 mL Bottle	1	4°C
Reagent F	50X Probenecid Solution[2]	4 mL Bottle	1	4°C
Reagent G	10X SLC Sodium Stimulus Buffer	10 mL Bottle	1	4°C

Getting Started

Before beginning your experiment, ensure you have all the necessary reagents and materials. ION Biosciences' Sodium-Dependent SLC Transporter Assay kit includes everything required for the assay, except for modulators, substrates, and SLC-expressing cell lines. A fluorescence plate reader with excitation capabilities between 485–525 nm and emission collection around 545 nm is also needed; ideally, the reader should support kinetic data collection at about 1 Hz. Examples of suitable instruments include the WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR, and Molecular Devices FlexStation.

These instructions are designed for both 96-well and 384-well microplate formats. Adjustments may be needed for other formats, such as 1536-well plates or non-adherent cells. ING-2 AM should be protected from direct light and solutions containing ING-2 AM should be used within 2 hours of preparation. We recommend seeding SLC-expressing cells in 96-well or 384-well plates 24 hours prior to the assay, with 20K cells per well for 96-well plates and 10K cells per well for 384-well plates.

Laboratory Procedures

1. Add 20 µL of DMSO (Reagent B) to the tube containing ING-2 AM (Reagent A).
2. Vortex until Reagent A is fully dissolved.
3. Add appropriate volume of water (Table 2, next page) to a 15 mL centrifuge tube.
4. Add 1 mL of 10X SLC Sodium Assay Buffer (Reagent D) to the tube from step 3.
5. Add 200 µL of 50X DySolv (Reagent C) to the tube from step 4.
6. Add 200 µL of 50X TRS[2] (Reagent E) to the tube from step 5.

Procedure Continues on Next Page

Laboratory Procedures (Continued)

7. If desired add 200 µL of 50X Probenecid[2] (Reagent F) to the tube from step 6.
8. Add 20 µL of ING-2 AM Solution from step 2 to the tube from step 7.
9. Briefly vortex the Dye Loading Solution from step 8 to mix well.

Table 2	Dye Loading Solution		
Label	**Name**	**Method A**	**Method B**
Reagent A+B	ING-2 AM solution in DMSO	20 µL	20 µL
Reagent C	50X DySolv	200 µL	200 µL
Reagent D	10X SLC Sodium Assay Buffer	1 mL	1 mL
Reagent E	50X TRS[2]	200 µL	200 µL
Reagent F	50X Probenecid[2]	200 µL	-
	Water	8.6 mL	8.8 mL
	Total	10 mL	10 mL

10. Completely remove the cell-culture medium from the 96-well or 384-well microplate containing the cells of interest.
11. Prepare the Wash Solution[3] in a 15 mL centrifuge tube by adding the appropriate amounts of water and 10X SLC Sodium Assay Buffer (Reagent D) as shown in Table 3 below. Briefly vortex to mix well.
12. Wash the cell-containing microplate once with Wash Solution[3]. 100 µL/well for 96-well plate and 20 µL/well for 384-well plate. Completely remove the Wash Solution[3] from the 96-well or 384-well plates.

Table 3	Wash Solution[3] (Optional)	
Label	**Name**	**Volume**
Reagent D	10X SLC Sodium Assay Buffer	1 mL
	Water	9 mL
	Total	10 mL

13. Add the Dye Loading Solution from step 9 to the cell-containing microplate. 100 µL/well for 96-well plate and 20 µL/well for a 384-well plate.
14. Incubate the cell-containing microplate with the Dye Loading Solution for 60 minutes at 37°C.

Procedure Continues on Next Page

Laboratory Procedures (Continued)

Note: If assaying SLC Transporter Modulators, continue with steps 15–17; otherwise, proceed to Step 18.

15. Prepare the Modulator Solution in a 15 mL centrifuge tube by adding the appropriate amounts of water, 10X SLC Sodium Assay Buffer (Reagent D), and 3X concentrated SLC transporter modulators as shown in Table 4 below. Briefly vortex to mix well.
16. Add the Modulator Solution from step 15 to the cell-containing microplate. 50 µL/well for a 96-well plate and 10 µL/well for a 384-well plate.
17. Incubate the cell-containing microplate for 15 minutes at 37°C.

Table 4	Modulator Solution (Optional)	
Label	**Name**	**Volume**
Reagent D	10X SLC Sodium Assay Buffer	1 mL
(Customer Supplied Material)	SLC Transporter Modulators	Enough for 3X concentration at total volume
	Water	Q.S. to total volume
	Total	10 mL

18. Prepare the Stimulus Solution[4] in a 15 mL centrifuge tube by adding the appropriate amounts of water, 10X SLC Sodium Stimulus Buffer (Reagent G), and 4X concentrated SLC transporter substrates as shown in Table 5 below. Briefly vortex the Stimulus Solution[4] to mix well.
19. Load the Stimulus Solution[4] from step 18 into a V-shaped reagent plate for assay readout. 100 µL/well for a 96-well plate and 20 µL/well for a 384-well plate.

Table 5	Stimulus Solution [4]	
Label	**Name**	**Volume**
Reagent G	10X SLC Sodium Stimulus Buffer	1 mL
(Customer Supplied Material)	SLC Transporter Substrates	Enough for 4X concentration at total volume
	Water	Q.S. to total volume
	Total	10 mL

20. Transfer the cell-containing microplate and reagent plate to a kinetic-imaging plate reader (e.g. WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR, or Molecular Devices FlexStation).

Procedure Continues on Next Page

Laboratory Procedures (Continued)

21. Acquire data using an excitation wavelength of ~515 nm, an emission wavelength of ~545 nm, and an acquisition frequency of ~1 Hz. Excitation sources commonly used for fluorescein, Fluo-4, and GFP (480-490 nm) are compatible with ION's ING-2 AM fluorescent dye. See Table 6 below for recommended instrument settings.
22. Begin baseline data acquisition for 30 seconds, then transfer the volume from the reagent plate to the cell-containing microplate. 50 µL/well for a 96-well plate and 10 µL/well for a 384-well plate. Continue data acquisition for an additional 270 seconds or longer.

Table 6	Recommended Instrument Settings
Setting	Recommendation
Read Mode	'Bottom' read mode only
Ex/Em wavelengths[5]	~515 nm/545 nm
Filter selection	GFP, FITC, YFP
Contact support@ionbiosciences.com for additional recommendations and guidance on optimizing your application.	

Laboratory Procedures - Footnotes

[1] DMSO is hygroscopic and should be stored tightly closed at -20° C with desiccant pack to prevent the solvent from becoming wet. Wet solvent causes difficulties with dissolution of the dye. Use the DMSO within 6 months of receipt.

[2] Caution is advised when using Probenecid and/or TRS as they may have undesirable effects on assay performance for the target of interest. Probenecid may be included in the Dye Loading Solution to aid in dye retention. This may be particularly important in certain cell lines (e.g. CHO cells). TRS contains a membrane-impermeant dye useful for masking extracellular fluorescence.

[3] We recommend washing the cells with wash solution only if residual culture medium remains in the well.

[4] The timing and volume of stimulus solution addition may vary. Some experiments may include the addition of other solutions to the cell-containing microplate prior to the addition of the stimulus solution. In these cases, the volume of the stimulus solution addition should be altered to account for the additional volume of solution in the cell-containing microplate.

[5] To prevent bleed-through or spectral overlap, the Ex/Em wavelengths may need to be optimized by broadening the interval between the wavelengths. Results generated on a Wavefront Panoptic.

Data Analysis

1. Plot the relative fluorescence units (F) over time as the raw kinetic fluorescence data (Figure 2).

2. Define the initial 30 seconds of relative fluorescence units (F) as the baseline. Average the baseline and define it as F_0. Calculate the ratio of F/F_0 at each time point. Plot F/F_0 over time as the baseline-normalized kinetic fluorescence data (Figure 3).

3. Use GraphPad® Prism (or another similarly capable graphing and statistical analysis software) to apply a linear regression to the first 30 seconds after stimulus addition on the baseline-normalized kinetic fluorescence data. Plot the slope or maximum velocity (V_{max}) of the linear regression against the substrate or modulator concentration to make a concentration response curve (Figure 4).

4. Alternatively, use GraphPad® Prism to apply an Area Under Curve (AUC) analysis on the baseline-normalized kinetic fluorescence data by measuring the area between the curve and the baseline at y=1. Plot the AUC against substrate or modulator concentration to make a concentration response curve (Figure 5).

5. Using GraphPad® Prism, run the non-linear regression "agonist vs response" on the concentration-response curve (V_{max} or AUC) to calculate the half-maximal effective concentration (EC_{50}). Alternatively, run the non-linear regression "inhibitor vs response" to calculate the half-maximal inhibitory concentration (IC_{50}) (Figure 6).

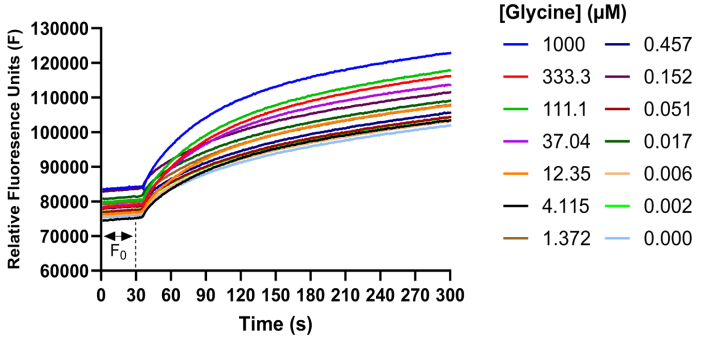

Figure 2. Raw Kinetic Fluorescence Data. Raw kinetic fluorescence data of SLC6A9-expressing HEK293T cells collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). Relative Fluorescence Units (F) were collected for 30 seconds before the stimulus addition of various concentrations of Glycine. After addition, the plate continued to be read for an additional 270 seconds.

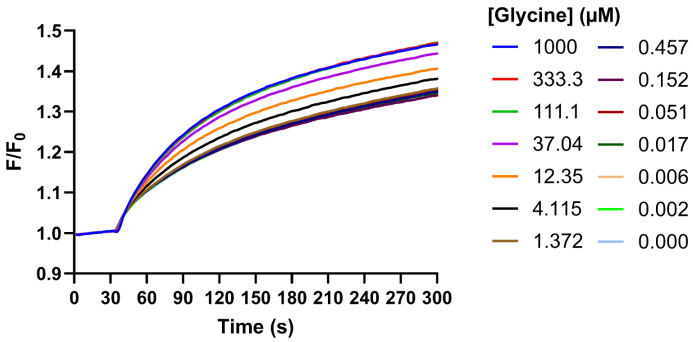

Figure 3. Baseline-Normalized Fluorescence Kinetic Data. Based on the Raw Kinetic Fluorescence Data from figure 2, average the Relative fluorescence Units (F) of the initial 30 seconds before stimulus addition as the baseline (F_0). Calculate the ratio of Relative fluorescence Units (F) over F_0 at each time point as F/F_0. Plot F/F_0 over time as the Baseline-Normalized Fluorescence Kinetic Data.

Data Analysis (Continued)

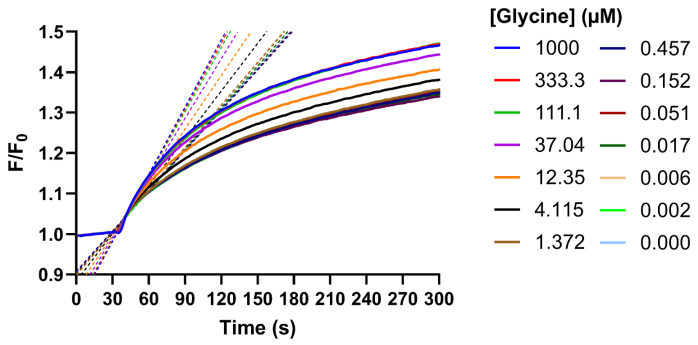

Figure 4. Maximum Velocity (V_{max}) Calculation. Based on the Baseline-Normalized Fluorescence Kinetic Data from Figure 3, conduct a linear regression on the first 30 seconds after stimulus addition. Calculate the slope and define it as the V_{max}.

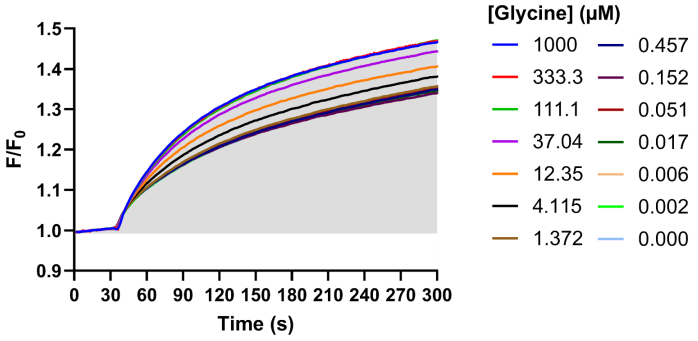

Figure 5. Area Under Curve (AUC) Calculation. Based on the Baseline-Normalized Fluorescence Kinetic Data from Figure 3, calculate the area between the curve and y=1 and define it as the AUC.

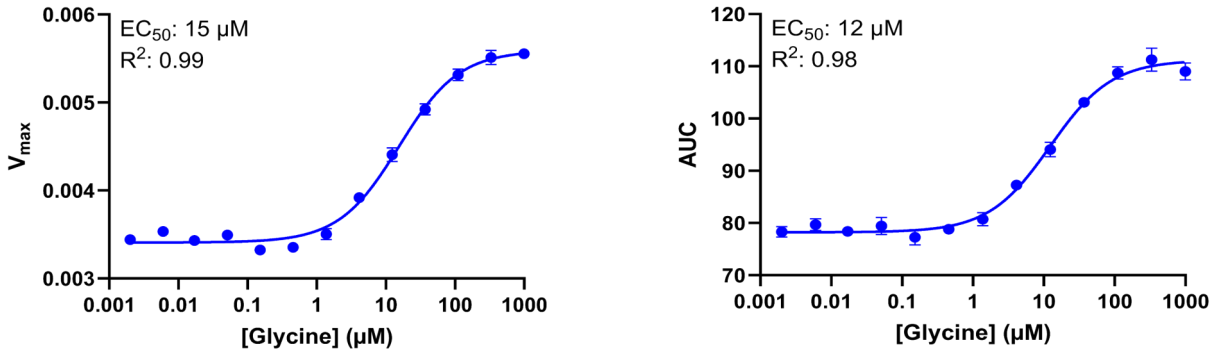

Figure 6. EC_{50} Calculation. Plot the V_{max} or the AUC against the compound concentrations and run the non-linear regression "agonist vs response" in GraphPad® Prism to calculate an EC_{50}. Alternatively, run the non-linear regression "inhibitor vs response" to calculate an IC_{50}.

Example Results

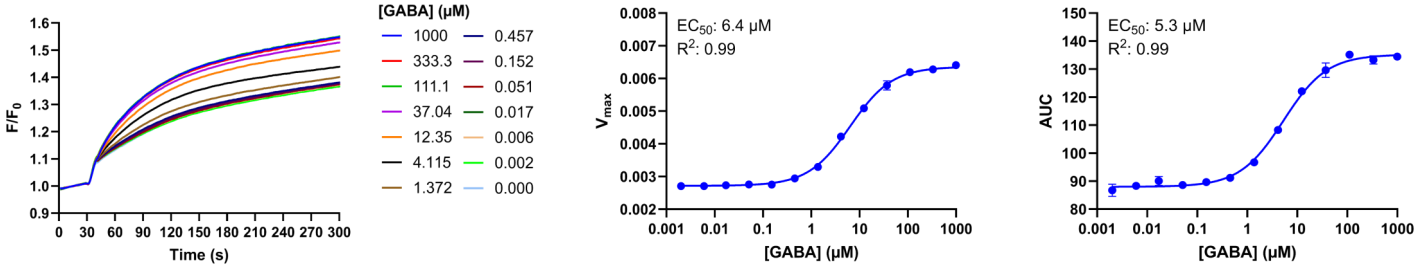

Figure 7. GABA Uptake in SLC6A1-Expressing HEK293T Cells. Baseline-normalized kinetic fluorescence data collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). GABA, the primary substrate for the SLC6A1 transporter, was added at 30 seconds. The V_{max} was calculated from the first 30 seconds following stimulus addition. The AUC was calculated between the curve and y=1. Error bars indicate the SEM (n = 3). The EC_{50} for GABA uptake is about 6 μM.

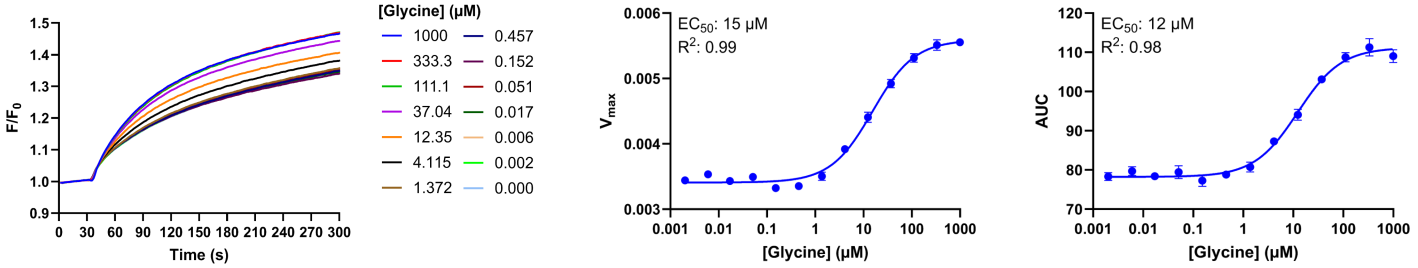

Figure 8. Glycine Uptake in SLC6A9-Expressing HEK293T Cells. Baseline-normalized kinetic fluorescence data collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). Glycine, the primary substrate for the SLC6A9 transporter, was added at 30 seconds. The V_{max} was calculated from the first 30 seconds following stimulus addition. The AUC was calculated between the curve and y=1. Error bars indicate the SEM (n = 3). The EC_{50} for Glycine uptake is about 15 μM.

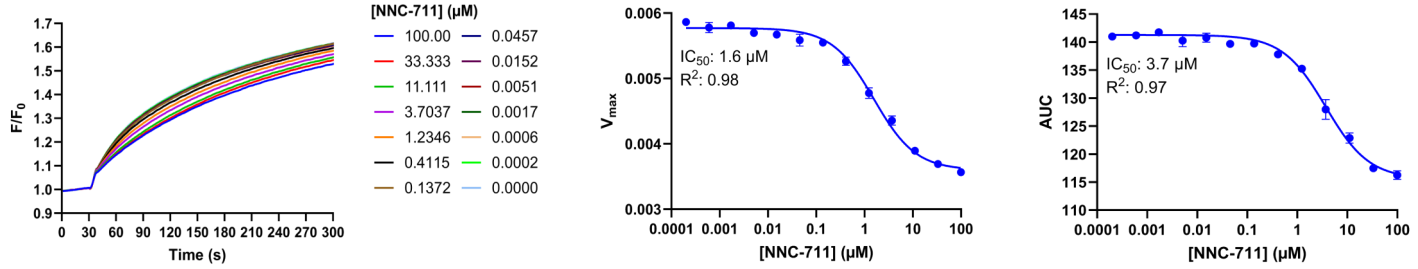

Figure 9. NNC-711 Inhibition of GABA Uptake in SLC6A1-Expressing HEK293T Cells. Baseline-normalized kinetic fluorescence data obtained using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). NNC-711, an inhibitor of the SLC6A1 transporter, was pre-incubated for 15 minutes before the start of the assay. GABA, the primary substrate for the SLC6A1 transporter, was added at 30 seconds at a concentration of 1 mM. The V_{max} was calculated from the first 30 seconds following stimulus addition. The AUC was calculated between the curve and y=1. Error bars indicate the SEM (n = 3). The IC_{50} for NNC-711 inhibition of GABA uptake is about 2 μM.

Example Results (Continued)

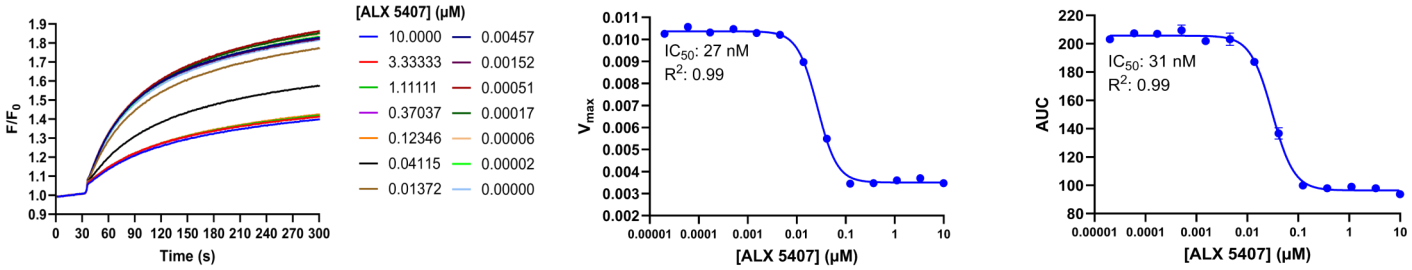

Figure 10. ALX 5407 Inhibition of Glycine Uptake in SLC6A9-Expressing HEK293T Cells. Baseline-normalized kinetic fluorescence data obtained using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). ALX 5407, an inhibitor of the SLC6A9 transporter, was pre-incubated for 15 minutes before the start of the assay. Glycine, the primary substrate for the SLC6A9 transporter, was added at 30 seconds at a concentration of 1 mM. The V_{max} was calculated from the first 30 seconds following stimulus addition. The AUC was calculated between the curve and y=1. Error bars indicate the SEM (n = 3). The IC_{50} for ALX 5407 inhibition of Glycine uptake is about 27 nM.

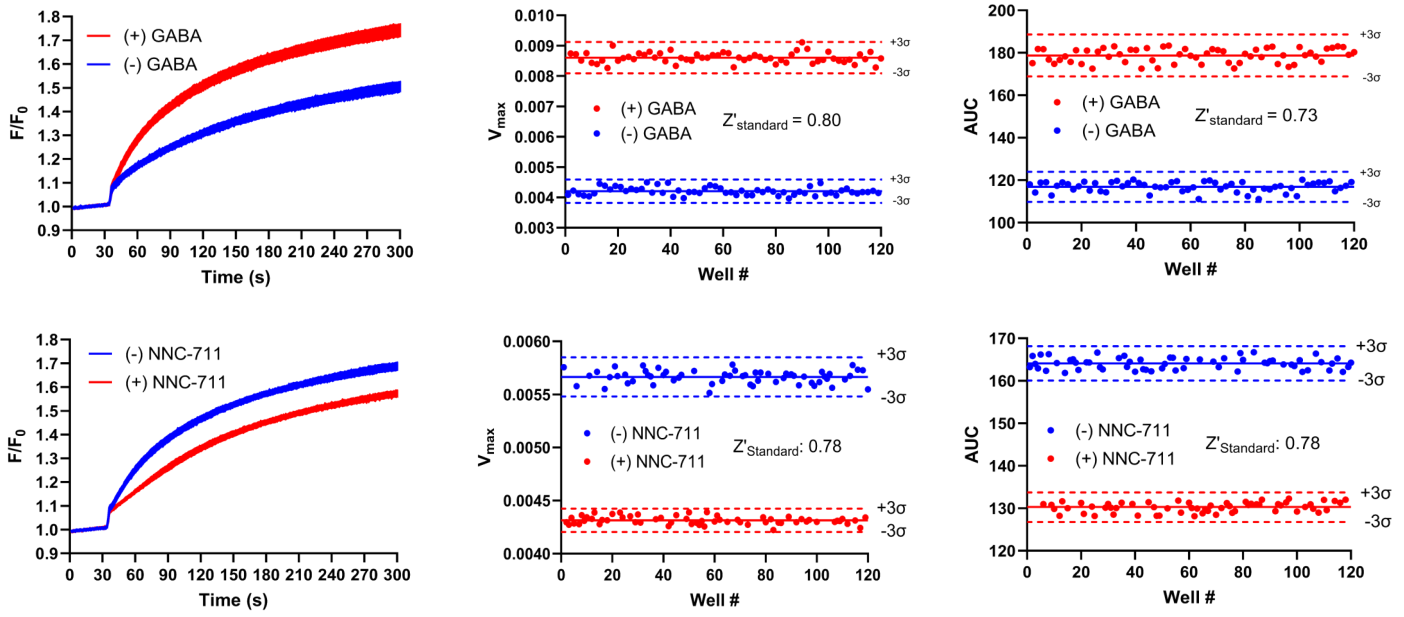

Figure 11. Z' Determination in SLC6A1-Expressing HEK293T Cells. Baseline-normalized kinetic fluorescence data collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). ±NNC-711, an inhibitor of the SLC6A1 transporter, was pre-incubated for 15 minutes at a concentration of 100 µM before the start of the assay. ±GABA, the primary substrate for the SLC6A1 transporter, was added at 30 seconds at a concentration of 1 mM. The V_{max} was calculated from the first 30 seconds following stimulus addition. The AUC was calculated between the curve and y=1. The Z' factor plots show a Z' above 0.7 for both GABA uptake and NNC-711 inhibition of GABA uptake (n = 60).

Additional Information

Additional dye indicator and buffer reagents can be purchased either directly from our website or by contacting our Sales Department. Custom and bulk sizes are also available. Contact Sales for more information.

Table 7	Additional Reagents	Available Sizes	
Kit Label	Name	Size	Catalog #
Reagent A	ING-2 AM	500 µg x 1 Vial 50 µg x 10 Vials 50 µg x 3 Vials	2011C 2011F 2011G
Reagent C	50X DySolv	20 mL Bottle	7501A
Reagent D	10X SLC Sodium Assay Buffer	Not a Regular Catalog Item. Contact Sales About Additional Purchase.	
Reagent E	50X TRS	20 mL Bottle	7060A
Reagent F	50X Probenecid	20 mL Bottle	7300P-50
Reagent G	10X SLC Sodium Stimulus Buffer	Not a Regular Catalog Item. Contact Sales About Additional Purchase.	

ION Assurance Policy

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